# IDENTIFICATION OF SARCOPLASMIC RETICULUM-LIKE SYSTEM IN *PHYSARUM POLYCEPHALUM*

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## 1. Introduction

Slime mold plasmodia of *Physarum polycephalum* represent a very primitive motile system. It has been established that the interaction of myosin and actin is responsible for the streaming of plasmodia protoplasm (reviewed [1–5]). Protoplasmic movement is regulated by the changes in the concentration of $Ca^{2+}$ [1–5]. Electron microscopic observations [6,7] have revealed that *Physarum plasmodia* contain an internal membrane system which has the ability to store calcium. Recently, the crude microsomal fraction capable to take up calcium was isolated from *Physarum* and partially characterized [8].

Here the microsomal fraction of *Physarum* was purified by the use of a sucrose-density gradient centrifugation and its properties were characterized. The results obtained provide evidence for the similarities between *Physarum* microsomal vesicles and the sarcoplasmic reticulum (SR) vesicles isolated from fast-twitch skeletal muscle.

## 2. Material and methods

Fresh starved plasmodia cultured on filter paper by the method in [9] as modified [10] were washed 2 times with distilled water. They were subsequently homogenized in a motor-driven glass–Teflon homogenizer, in a buffer containing 20 mM imidazole (pH 7.2), 100 mM KCl, and 1 mM β-mercaptoethanol.

The crude microsomal fraction sedimenting between 8000 and 50 000 × *g*, suspended in 250 mM

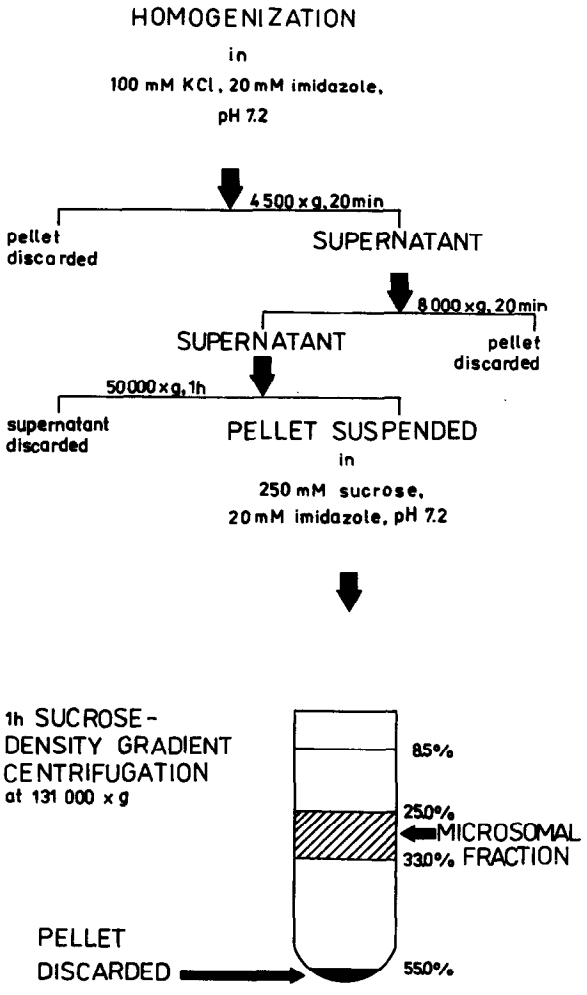


Fig.1. Procedure of the fractionation of the microsomal fraction from *Physarum polycephalum*.

sucrose and 10 mM imidazole (pH 7.2) (buffer A), was applied on a continuous sucrose-density gradient (8.5–55.0% sucrose in 10 mM imidazole (pH 7.2)) and subsequently centrifuged for 1 h at 131 000 × *g* in a Beckman L5-50 ultracentrifuge. Two fractions were obtained by this procedure: pellet and the fraction, which formed a band at the level of 25–33% sucrose (fig.1). The latter one, defined as the microsomal fraction, was suspended in the buffer A and characterized in the present work.

In order to inhibit the activities of proteolytic enzymes, the purification procedure was carried out in the presence of 50 000 KIU/l Trasylol (Polfa, Jelenia Góra).

The SR vesicles were obtained from fast-twitch rabbit skeletal muscle by the method in [11].

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–gel electrophoresis) in 7.5% slab-gel was carried out according to [12]. Protein content was determined according to [13] and phospholipid phosphorus content [14]. Total lipids were extracted from the vesicles according to [15]. Individual phospholipids were separated by silica-gel thin-layer chromatography. A mixture of chloroform, methanol and water (65:25:4, by vol.) was used as a solvent.

Double diffusion test in agarose plates was performed according to [16]. The specificity of the antibody has been checked in [17,18].

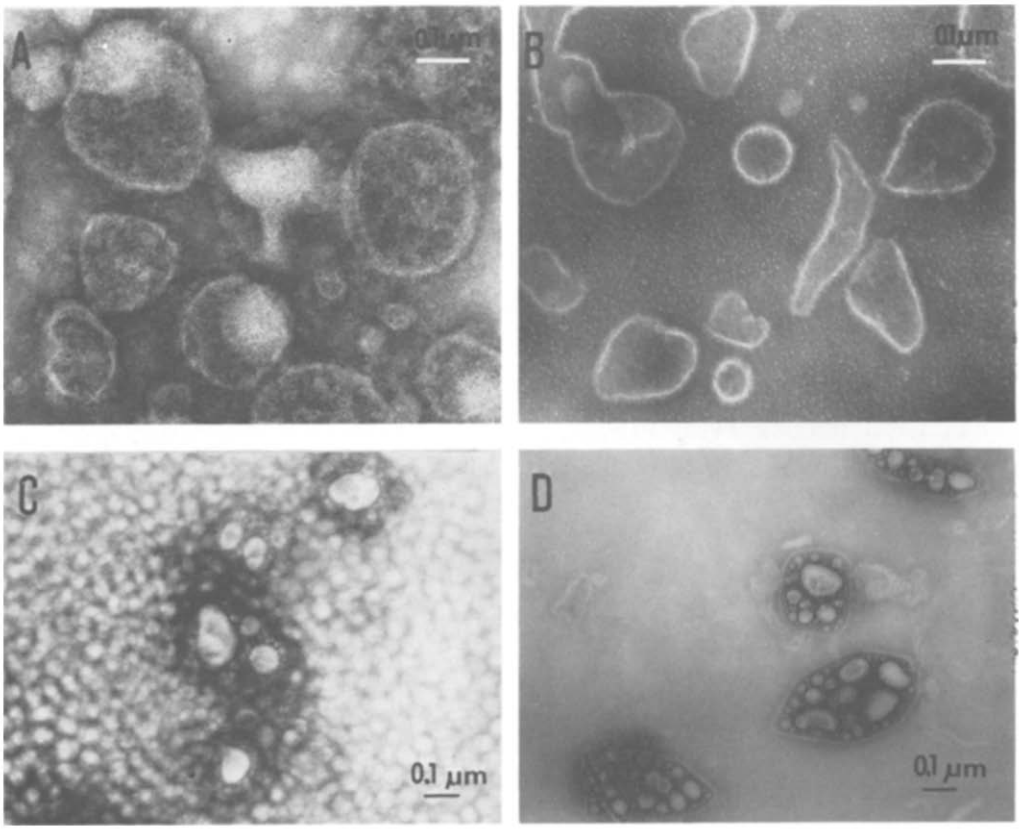


Fig.2. Electron micrographs of *Physarum* microsomal vesicles (A,C) and SR vesicles (B,D). Vesicles were negatively stained with uranyl acetate (A,C) or ammonium molybdate (B,D). (A,B) original vesicles; (C,D) vesicles loaded with the deposits of calcium oxalate. For loading with calcium oxalate the microsomal vesicles (800 μg protein in 4 ml buffer) were incubated for 30 min at 25°C in a buffer containing 10 mM ATP, 10 mM $MgCl_2$, 10 mM oxalate, 100 μM KCl and 10 mM imidazole (pH 7.2). Portions (4 μl) of 100 mM $CaCl_2$ were added to the incubation mixture at 5 min intervals. Subsequently, the suspension was layered on the top of the continuous sucrose-density gradient and centrifuged for 1 h under the conditions in section 2. The pellet obtained by this procedure was used in the further studies.

Microsomal vesicles were negatively stained with 2% ammonium molybdate or 2% uranyl acetate and examined in a JEM 100 B electron microscope at an accelerating voltage of 80 kV.

## 3. Results and discussion

Negatively stained *Physarum* microsomal fraction consists of the spherical membraneous vesicles resembling those of SR isolated from fast-twitch skeletal muscle (fig.2A,B). Mitochondria or filaments of contractile proteins have not been observed in the preparations.

The analysis of total phospholipid phosphorus shows that the phospholipid content in the *Physarum* microsomal vesicles and in the SR vesicles is similar. Phospholipid to protein ratio in both kinds of the vesicles is ~0.7 (w/w). As shown by thin-layer chromatography (fig.3), phosphatidylcholine and phosphatidylethanolamine are the major phospholipids in both, *Physarum* microsomal vesicles and SR vesicles. In addition, vesicles obtained from plasmodia contain considerable amounts of cardiolipid, which is not present in the SR vesicles. Lipid composition of plasmodia microsomal vesicles differs from that of SR vesicles also with respect to the higher content of cholesterol and other neutral lipids (fig.3).

The crude *Physarum* microsomal fraction (sedimenting between 8000 and 50 000 × *g*) contains several protein bands ranging from mol. wt 200 000–18 000 (fig.4A). The pellet obtained upon subsequent fractionation on a sucrose-density gradient contains most of these bands, including mol. wt 200 000 and 42 000 bands, which most probably originate from the contaminations with myosin and actin (fig.4B). The protein composition of the microsomal fraction is very simple and in this respect similar to the SR vesicles (fig.4C,D). In agreement with [8] the main protein of *Physarum* microsomal vesicles has the mol. wt 105 000. This protein accounts for ~50% of total membraneous protein. Few other proteins are present in the microsomal vesicles in lower amounts.

Ouchterlony double diffusion test indicates that the microsomal fraction from *Physarum* dissolved in 1% Triton X-100 crossreacts with the antibody against $Ca^{2+}$, $Mg^{2+}$-dependent ATPase from the SR vesicles (fig.5). This result suggests the immunological identity between the mol. wt 105 000 protein from *Physarum* vesicles and the $Ca^{2+}$, $Mg^{2+}$-dependent ATPase from SR membranes. It is worth mentioning that there is no crossreactivity between the antibody against calsequestrin, the SR calcium-binding protein, and any of the proteins present in the Triton extract of the microsomal fraction from *Physarum* (fig.5).

The microsomal fraction obtained from *Physarum* by differential centrifugation was reported [8] to

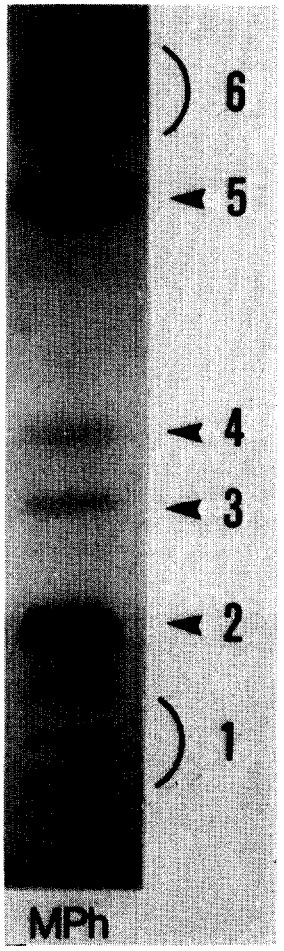


Fig.3. Lipid composition of *Physarum* microsomal vesicles. Lipids extracted from the vesicles according to [14] were separated by thin-layer chromatography with chloroform/methanol/water (65:25:4, by vol.) as a solvent. (MPh) lipid extract from *Physarum* microsomal vesicles; (1) lysophosphatidylcholine, phosphatidylinositol, phosphatidylserine; (2) phosphatidylcholine; (3) phosphatidylethanolamine; (4) unidentified; (5) cardiolipin; (6) neutral lipids.

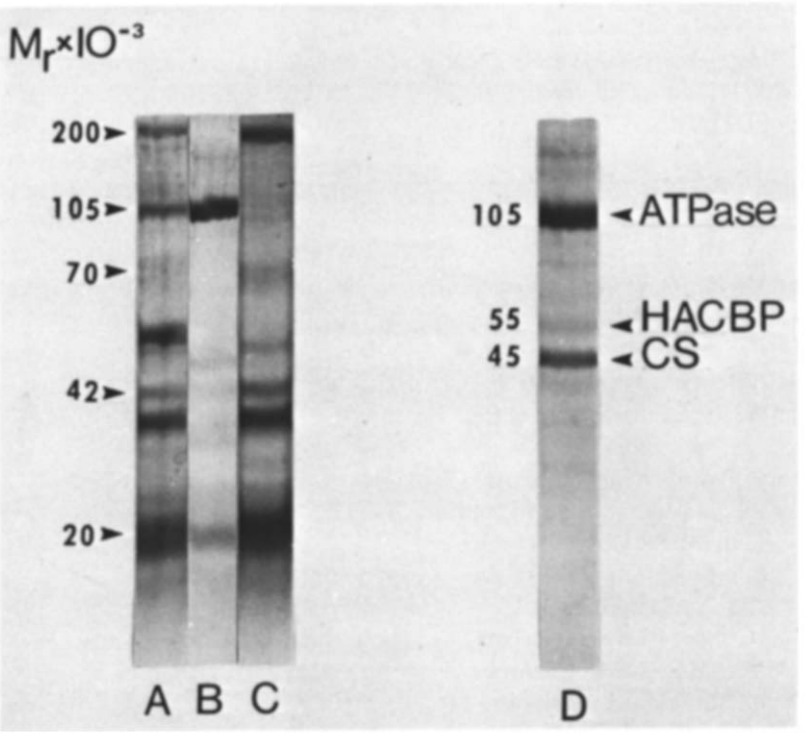


Fig.4. Protein composition of *Physarum* microsomal subfractions. (A) crude microsomal fraction sedimenting between 8000 and 50 000 × *g*; (B) microsomal fraction found at the level of 25–33% sucrose; (C) pellet after sucrose-density gradient centrifugation; (D) SR vesicles. SDS–gel electrophoresis was carried out according to [11]. (CS) calsequestrin; (HACBP) high affinity calcium binding protein.

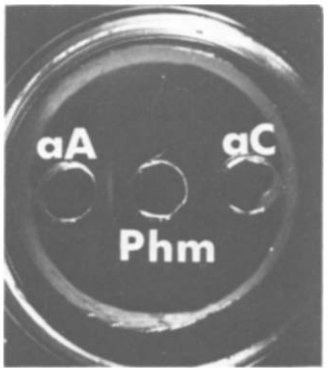


Fig.5. Ouchterlony double diffusion test in agarose plate. The well labeled 'aA' contained the antiserum from rabbit immunized against the $Ca^{2+}$, $Mg^{2+}$-dependent ATPase from rat SR; the well labeled 'Phm' contained the Triton X-100 extract of the microsomal fraction of *Physarum*; the well labeled 'aC' contained the antiserum from sheep immunized against calsequestrin from rabbit SR.

exhibit $Ca^{2+}$, $Mg^{2+}$-dependent ATPase activity and the ability to take up calcium in the presence of ATP. However, the addition of oxalate to the incubation mixture did not have any effect on the calcium uptake. Therefore in the present work the effect of oxalate was re-examined. For this purpose an attempt was made to load *Physarum* vesicles with calcium oxalate in the presence of ATP. The vesicles incubated in the loading medium were subsequently fractionated on the sucrose-density gradient. Negative staining reveals that the pellet obtained by this procedure consists of the vesicles containing deposits of calcium oxalate. This indicates that *Physarum* vesicles accumulate calcium in the presence of ATP and that these vesicles are permeable to oxalate in the way similar to the SR vesicles.

It has been reported for various non-muscle systems (reviewed [1–5]) that motility of cells requires the presence of calcium and is regulated by the changes in

calcium concentration in the cytoplasm. The membraneous fraction able to take up calcium and therefore most probably representing a calcium-sequestering system has been recently isolated from various cells and partially characterized [19–25].

The results in [6,7] have indicated that *Physarum* contains the vacuoles able to store deposits of calcium oxalate. It is reasonable to assume that the vesicles isolated and characterized in this work originate from these structures. The present findings suggest that an intracellular membrane system analogous to the SR of skeletal muscle is present in slime mold plasmodia of *Physarum polycephalum.*

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